

Occurrence and cultural features of *Streptococcus milleri* in various body sites

PAULINE M. POOLE AND GEORGE WILSON

From the Public Health Laboratory, Chester City Hospital, Chester CH2 3EG, UK

SUMMARY An investigation was made into the habitat of commensal strains of *Streptococcus milleri*. These showed distinctive patterns of cultural features, dependent on their sites of origin, which were compared with those prevalent in strains grown from the appendix, 'anal' lesions, and Bartholin's abscesses. A biotype, which showed a marked affinity for the vagina and produced acid from raffinose and melibiose, was identified.

Streptococcus milleri is an important cause of purulent disease, being the streptococcus most often isolated from such lesions in internal organs (Parker and Ball, 1976). Described originally in abscesses of the oral cavity (Guthof, 1956), it has been found in abscesses of the liver (Bateman *et al.*, 1975; Reid and Davidson, 1976) and in the central nervous system (De Louvois *et al.*, 1977; Melo and Raff, 1978) as well as in appendicitis (Poole and Wilson, 1977), peritonitis, empyema, meningitis, and other pus-forming lesions (Parker and Ball, 1976). The presence of group F streptococci, which were probably *Strep. milleri*, has likewise been observed on many occasions in similar lesions (Thomas, 1939; Rantz, 1942; Wheeler and Foley, 1943; Foley, 1947; Koepke, 1965; Wort, 1975; Bannatyne and Randall, 1977). The anatomical distribution of disease associated with these streptococci suggests that a number originate in the gut (Wort, 1975; Parker and Ball, 1976), an observation supported by the isolation of *Strep. milleri* from the normal as well as the inflamed appendix (Poole and Wilson, 1977), and of group F streptococci from faeces (Hare and Maxted, 1935). Their presence on teeth (Phillips *et al.*, 1976; Mejäre and Edwardsson, 1975) and in dental root canals (Ottens and Winkler, 1962) is a potential source of dental sepsis, which in turn has been related to the development of brain abscess (Ingham *et al.*, 1978). Group F streptococci have been observed in the nasopharynx (Hare, 1935), in the throat (Long *et al.*, 1934), and in the vagina (Lancefield and Hare, 1935; Wort, 1975). Information on the occurrence of *Strep. milleri* is, however, by no means complete and the subject merits further investigation.

Methods

Specimens examined for the presence of *Strep. milleri* included extracted teeth obtained from 52 patients, 25 of whom had normal teeth and 27 dental caries; 71 throat swabs, which had yielded a normal flora on routine examination and which originated from persons in whom there had been no recent treatment with antibiotics; 225 unselected vaginal swabs; and faeces or rectal swabs from 184 persons. One hundred and fifty-two of the specimens of faecal origin were obtained during screening for the carriage of gastrointestinal pathogens; these formed a normal group. The remaining 32 specimens were from patients suffering from peritonitis, resultant on varying abdominal pathology.

All specimens were inoculated into Todd-Hewitt broth containing 7% lysed horse blood, 30 mg/l nalidixic acid, and 50 mg/l sulphadimidine. The inoculated broths were incubated in 10% CO₂ in air at 37°C for 24 hours and then subcultured to blood agar and to neomycin blood agar (50 mg/l neomycin) for further similar incubation. The plates were examined, and colonies suspected of being *Strep. milleri* were subcultured for purity and identified by methods previously described (Poole and Wilson, 1976; 1977). The isolates obtained were compared with strains of *Strep. milleri* cultured during the course of routine microbiological examination and similarly characterised—from four dental abscesses, 26 appendices, 19 purulent lesions adjacent to the anus, and three Bartholin's abscesses.

In addition to the characterisation tests used in our previous investigation, 170 strains of *Strep. milleri* were examined for the ability to produce acid from melibiose and for the production of hyaluronidase. The presence of hyaluronidase was determined

using the method of Oakley and Warrack (1951), substituting bovine for horse synovial fluid.

Results

The enrichment broth used to encourage the growth of *Strep. milleri* was of most value in the examination of faecal specimens, in which it largely eliminated Gram-negative bacteria and allowed *Strep. milleri*, if present, to multiply more or less unimpeded, subculture frequently producing abundant growth. This was especially marked in specimens originating from patients with peritonitis. *Strep. milleri* was easily distinguished from sulphonamide-resistant enterococci likewise enriched by the broth. The broth was less effective, although still useful, in the culture of vaginal swabs, *Strep. milleri* generally being present in only very small numbers on subculture compared with enterococci which grew profusely. In the examination of teeth and throat swabs, the irrelevant flora was diminished only marginally, the growth of other small streptococci resistant to sulphonamide being similarly enhanced by the enrichment method used. Careful search of subcultures was required to distinguish the relevant colonies.

Table 1 Number and percentage of isolates of *Strep. milleri* by source, subdivided by age

<i>Source</i>		<i>No. of patients</i>	<i>Strep. milleri</i>	
			<i>No.</i>	<i>%</i>
Teeth				
Normal	All ages	25	7	28.0
Caries	All ages	27	10*	37.0
Throat				
	All ages	71	22	31.0
	< 5 yr	15	0	
	5-14 yr	12	3	25.0
	> 15 yr	44	19	43.2
Faeces				
	All ages	160	24	15.0
Normal				
	< 5 yr	31	0	
	5-14 yr	13	2	15.4
	> 15 yr	116	22	18.9
	All ages	32	15	46.9
Peritonitis				
	All ages	225†	41	18.2
Vagina				
	15-44 yr	154	25	16.2
	> 45 yr	40	11	27.5

* 1 patient, 2 strains.

† 31 age unknown.

Table 1 shows the distribution of *Strep. milleri* at various sites. There was no significant difference in isolation rate between patients with normal teeth and with dental caries. The occurrence of *Strep. milleri* in throat and faeces is shown in three age groups. When isolates in the < 5 and 5-14 year age groups are combined, the difference between them and the > 15 group is significant for isolates from both throats ($P < 0.01$) and faeces ($P < 0.05$).

The difference is due almost entirely to the absence of positives in those under 5 years, rates in the other age groups not being significantly different. The very much more frequent presence of *Strep. milleri* in faeces of patients with peritonitis compared with the normal group is highly significant ($P < 0.001$). There was no significant difference in isolation rate from the vagina by age. *Strep. milleri* occurred twice as frequently in the female throat (36.0%) as in the male (19.0%). Sex did not, however, affect the isolation rate from teeth or faecal material.

In all, 172 strains of *Strep. milleri* were available for investigation (Table 2). They included 120 isolates from sites specifically examined for its presence, and 52 isolates made from material routinely submitted to the laboratory for culture. Nine (5.2%) strains carried the antigen of group A, 12 (7.0%) group C, 83 (48.3%) group F, and 15 (8.7%) group G. Fifty-three (30.8%) were non-groupable with these antisera. Both groupable and non-groupable *Strep. milleri* were widely distributed throughout the various sites. Strains of groups A and G each accounted for one-fifth of the isolates from the throat. Group F streptococci predominated in teeth, faeces, anal lesions, and vagina. Group F strains were, however, found in only one-quarter of the appendices and seldom in the throat. At these two sites non-groupable strains prevailed.

Approximately one-quarter of all isolates were haemolytic. Haemolytic strains occurred most commonly in teeth, throat, and anal lesions. Their prevalence in teeth and throat may have been influenced by the relative difficulty in identifying *Strep. milleri* at these sites, compared with the sites for which the selective methods used were rather better suited.

Just over one-quarter of the isolates of *Strep. milleri* produced acid from both raffinose and melibiose. The raffinose+/melibiose+ biotype (R+/M+) accounted for the majority of strains from the vagina, and all those found in Bartholin's abscesses. One-quarter of the faecal strains were likewise R+/M+. This characteristic was absent in isolates from the throat and appendix. Strains giving the R+/M+ reaction were found in group F, group G, and non-groupable strains (Table 3), and occurred three times more frequently in non-haemolytic than in haemolytic strains.

Table 3 also shows the strongly positive association between the production of hyaluronidase and haemolysis, this being most marked in groups containing the A, C, and F antigens. This finding reflects the observation (Colman and Williams, 1972) that haemolytic strains of group F produce this enzyme, while it is unusual for non-haemolytic *Strep. milleri* to do so.

Table 2 *Some cultural features of 172 strains of Strep. milleri and percentage of strains giving stated reaction, by site of isolation*

	Teeth (n = 18)	Dental abscess (n = 4)	Throat (n = 22)	Normal faecal (n = 24)	Peritonitis faecal (n = 15)	'Anal' lesions (n = 19)	Appendix (n = 26)	Vagina (n = 41)	Bartholin's abscess (n = 3)	All sites (n = 172)
Lancefield group										
A	1(5.5)	0	4(18.2)	1(4.2)	1(6.6)	1(5.2)	1(3.8)	0	0	9(5.2)
C	0	0	3(13.6)	2(8.3)	2(13.3)	2(10.5)	2(7.7)	1(2.4)	0	12(7.0)
F	12(66.6)	1(25.0)	3(13.6)	11(45.8)	7(46.6)	11(57.9)	7(26.9)	29(70.9)	2(66.6)	83(48.2)
G	0	1(25.0)	4(18.2)	0	1(6.6)	4(21.0)	3(11.5)	1(2.4)	1(33.3)	15(8.7)
Non-groupable	5(27.7)	2(50.0)	8(36.4)	10(41.6)	4(26.6)	1(5.2)	13(50.0)	10(24.4)	0	53(30.8)
Haemolytic	12(66.6)	2(50.0)	9(40.9)	6(25.0)	5(28.2)	8(42.1)	3(11.5)	2(4.9)	0	47(27.3)
Non-haemolytic	6(33.3)	2(50.0)	13(59.0)	18(75.0)	10(66.6)	11(57.9)	23(88.5)	39(95.1)	3(100)	125(72.6)
Acid production from raffinose + melibiose	1(5.5)	1(25.0)	0	6(26.1)	4(26.6)	2(10.5)	0	30(73.2)	3(100)	47(27.3)
*Pattern of main features	HAEM F ng	—	haem ng	haem f ng r+/m+	haem f ng r+/m+	haem F	f NG	F R+/M+	F R+/M+	

*Upper case: 50% + strains show features. Lower case: 25-49% strains show features.

F = group F; Ng = non-groupable; R+/M+ = acid production raffinose + melibiose; HAEM = haemolytic.

Table 3 *Acid production from raffinose and melibiose and production of hyaluronidase in 170* strains of Strep. milleri. Numbers and percentages of strains giving stated reaction by Lancefield groups and haemolytic reaction*

	Lancefield group									
	A (n = 9)		C (n = 11)		F (n = 82)		G (n = 15)		Non-groupable (n = 53)	
	H	NH	H	NH	H	NH	H	NH	H	NH
Total strains	2	7	3	8	20	62	7	8	13	40
R+/M+	0	0	0	0	2	32	3	1	0	9
					(10.0)	(51.6)	(42.8)	(12.5)		(22.5)
Hyaluronidase production	2	0	3	0	15	1	2	10	16	30
	(100)		(100)		(75.0)	(1.6)	(25.0)	(76.9)	(40.0)	(66.6)

*Two strains did not grow well enough for results to be included.

H = haemolytic; NH = nonhaemolytic.

Percentages are given in parentheses.

Discussion

We have found *Strep. milleri* in 20% overall of potential carrier sites investigated, indicating that it is a widely distributed human commensal. Parker and Ball (1976) observed a tendency for isolates of *Strep. milleri* from patients with bacteraemia or purulent lesions to occur more frequently in the male, and to become more numerous with increasing age. We have been unable to demonstrate any consistent association between sex and colonisation at the various sites, and have found no significant difference in the occurrence of these streptococci between older children and adults. This is similar to our previous finding (Poole and Wilson, 1977) that their occurrence in the appendix is independent of age. Their absence from children under 5 years in the present study is, however, consistent with the

rarity of their isolation from lesions in very young babies (Parker and Ball, 1976).

We have identified *Strep. milleri* in 31% of all throat swabs, a result which differs considerably from the findings of Guthof (1956), who was unable to demonstrate these streptococci in the nasopharynx, and of Mejäre and Edwardsson (1975), who grew them from less than 1.0% of material sampled from the oral cavity. However, the nine haemolytic strains described here represent an isolation rate of 12%, resembling the observation of Long *et al.* (1934), who found haemolytic strains in 6-8% of normal throats. It far outstrips our own previously estimated isolation rate of 0.6% haemolytic strains at this site (Poole and Wilson, 1976). Likewise the three strains of group F found by us indicate an occurrence in the throat 100 times more frequent than that noted by Wort (1975).

One-third of the teeth examined in this study yielded *Strep. milleri*, a finding consistent with the work of Mejäre and Edwardsson (1975), who grew these streptococci in up to 50% of material from the gingival pocket and in 25% of that from supra-gingival plaque.

There is little information with which to compare our isolation rates from normal faeces other than the observation of Hare and Maxted (1935) that haemolytic strains of group F occurred in the faeces of 5% of normal puerperal women.

Although the presence of *Strep. milleri* has been noted in the vagina previously, there are no isolation rates available with which to compare our results. While we were unable to demonstrate any significant difference in occurrence at this site between younger and older women, our results showed a trend towards increased colonisation with age. The present study, however, confirms our previous observation (Poole and Wilson, 1976) of the rarity of haemolytic strains at this site.

A consideration of the cultural characteristics of streptococci from the different sites reveals that some features occur more frequently than others, depending on the origin of the strains. They form patterns (Table 3), which are distinct for each site and which may in theory provide a clue as to the source of streptococci found in purulent lesions. The outstanding feature is the occurrence of R+/M+. The presence of this biotype directs attention primarily to the vagina, where the majority of strains are non-haemolytic, group F, and R+/M+. The culture of R+/M+ from Bartholin's abscess is consistent with a vaginal source of infection on both anatomical and biochemical grounds. Strains found in 'peritonitis' faecal material likewise resemble those occurring in normal faeces. Most are non-haemolytic, of either group F or non-groupable, and a similar percentage are R+/M+.

The increased isolation rate from faeces in the presence of peritonitis parallels the increase found in the inflamed appendix when compared with the normal organ (Poole and Wilson, 1977). The interpretation of this finding is obscure. Possibly it is a result, rather than a cause, of inflammation, since on checking records of individual patients, we found that strains of *Strep. milleri* isolated from faeces frequently differed from those in the peritoneal lesion in both Lancefield group and cultural features. Sometimes *Strep. milleri* were not even cultured from the corresponding peritoneal lesion. It might be expected that isolates from the appendix and from 'anal' lesions would likewise reflect those found in the faeces. This was not always so.

Strains from 'anal' lesions were more commonly haemolytic, and while group F predominated,

non-groupable strains were rarely found. On the other hand, group F streptococci formed only one-quarter of the strains grown from the appendix, while here, as in our previous investigation (Poole and Wilson, 1977), non-groupable strains accounted for half the isolates. The biotype R+/M+ was present only occasionally in 'anal' lesions and was not found in the appendix.

There were too few dental abscesses for a comparison of streptococci isolated from such lesions with strains found in teeth. Here the majority were haemolytic and group F abounded, whereas in the anatomically adjacent throat fewer were haemolytic, group F occurred infrequently, and the strains were more evenly distributed throughout all the groups. Almost half of our strains which carried group A antigen were grown from the throat, leading one to speculate that any isolations of group A from purulent lesions may well have had their origin at this site. However, despite the existence of characteristic patterns of cultural features in the various sites, the differences are not so marked between individual strains, with the result that any conclusion as to the original source of infection must inevitably be tenuous.

We thank Mr J. Lobb, of the Epidemiological Research Laboratory, Colindale, for statistical assistance.

References

- Bannatyne, R. M., and Randall, C. (1977). Ecology of 350 isolates of group F streptococcus. *American Journal of Clinical Pathology*, **67**, 184-186.
- Bateman, N. T., Eykyn, S. J., and Phillips, I. (1975). Pyogenic liver abscess caused by *Streptococcus milleri*. *Lancet*, **1**, 657-659.
- Colman, G., and Williams, R. E. O. (1972). Taxonomy of some human viridans streptococci. In *Streptococci and Streptococcal Diseases*, edited by L. W. Wannamaker and J. M. Matsen, pp. 281-299. Academic Press, London and New York.
- De Louvois, J., Gortvai, P., and Hurley, R. (1977). Bacteriology of abscesses of the central nervous system; a multicentre prospective study. *British Medical Journal*, **2**, 981-984.
- Foley, G. E. (1947). Further observations on the occurrence of streptococci of groups other than A in human infection. *New England Journal of Medicine*, **237**, 809-811.
- Guthof, O. (1956). Ueber pathogene "vergrünende Streptokokken": Streptokokken-Befunde bei dento-genen Abszessen und Infiltraten im Bereich der Mundhöhle. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I Abteilung: Originale*, **166**, 553-564.
- Hare, R. (1935). The classification of haemolytic streptococci from the nose and throat of normal human

- beings by means of precipitin and biochemical tests. *Journal of Pathology and Bacteriology*, **41**, 499-512.
- Hare, R., and Maxed, W. R. (1935). The classification of haemolytic streptococci from the stools of normal pregnant women and of cases of scarlet fever by means of precipitin and biochemical tests. *Journal of Pathology and Bacteriology*, **41**, 513-520.
- Ingham, H. R., High, A. S., Kalbag, R. M., Sengupta, R. P., Tharaggonnet, D., and Selkon, J. B. (1978). Abscesses of the frontal lobe of the brain secondary to covert dental sepsis. *Lancet*, **2**, 497-499.
- Koepeke, J. A. (1965). Meningitis due to *Streptococcus anginosus* (Lancefield group F). *Journal of the American Medical Association*, **193**, 739-740.
- Lancefield, R. C., and Hare, R. (1935). The serological differentiation of pathogenic and non-pathogenic strains of hemolytic streptococci from parturient women. *Journal of Experimental Medicine*, **61**, 335-349.
- Long, P. H., Bliss, E. A., and Walcott, C. F. (1934). Studies upon minute hemolytic streptococci 2. The distribution of minute hemolytic streptococci in normal and diseased human beings. *Journal of Experimental Medicine*, **60**, 633-641.
- Mejäre, B., and Edwardsson, S. (1975). Observations on the characteristics of *Streptococcus milleri* and its occurrence as a regular inhabitant of the human mouth (abstract). *Caries Research*, **9**, 308.
- Melo, J. C., and Raff, M. J. (1978). Brain abscess due to *Streptococcus MG.-intermedius* (*Streptococcus milleri*). *Journal of Clinical Microbiology*, **7**, 529-532.
- Oakley, C. L., and Warrack, G. H. (1951). The ACRA test as a means of estimating hyaluronidase, deoxyribonuclease and their antibodies. *Journal of Pathology and Bacteriology*, **63**, 45-55.
- Ottens, H., and Winkler, K. C. (1962). Indifferent and haemolytic streptococci possessing group-antigen F. *Journal of General Microbiology*, **28**, 181-191.
- Parker, M. T., and Ball, L. C. (1976). Streptococci and aerococci associated with systemic infection in man. *Journal of Medical Microbiology*, **9**, 275-302.
- Phillips, I., Warren, C., Harrison, J. M., Sharples, P., Ball, L. C., and Parker, M. T. (1976). Antibiotic susceptibilities of streptococci from the mouth and blood of patients treated with penicillin or lincomycin and clindamycin. *Journal of Medical Microbiology*, **9**, 393-404.
- Poole, P. M., and Wilson, G. (1976). Infection with minute-colony-forming β -haemolytic streptococci. *Journal of Clinical Pathology*, **29**, 740-745.
- Poole, P. M., and Wilson, G. (1977). *Streptococcus milleri* in the appendix. *Journal of Clinical Pathology*, **30**, 937-942.
- Rantz, L. A. (1942). Streptococcal meningitis: four cases treated with sulfonamides in which the etiological agent was an unusual streptococcus. *Annals of Internal Medicine*, **16**, 716-726.
- Reid, T. M. S., and Davidson, A. I. (1976). *Streptococcus milleri* liver abscesses (Letter). *Lancet*, **1**, 648-649.
- Thomas, A. (1939). Purulent meningitis produced by the minute hemolytic streptococcus (Long and Bliss) and B. coli. *Journal of the Mount Sinai Hospital*, **5**, 702-704.
- Wheeler, S. M., and Foley, G. E. (1943). A note on non-group-A streptococci associated with human infection. *Journal of Bacteriology*, **46**, 391-392.
- Wort, A. J. (1975). Observations on group-F streptococci from human sources. *Journal of Medical Microbiology*, **8**, 455-457.

Requests for reprints to: Dr Pauline M. Poole, Director, Public Health Laboratory, Chester City Hospital, Hoole Lane, Chester CH2 3EG, UK.